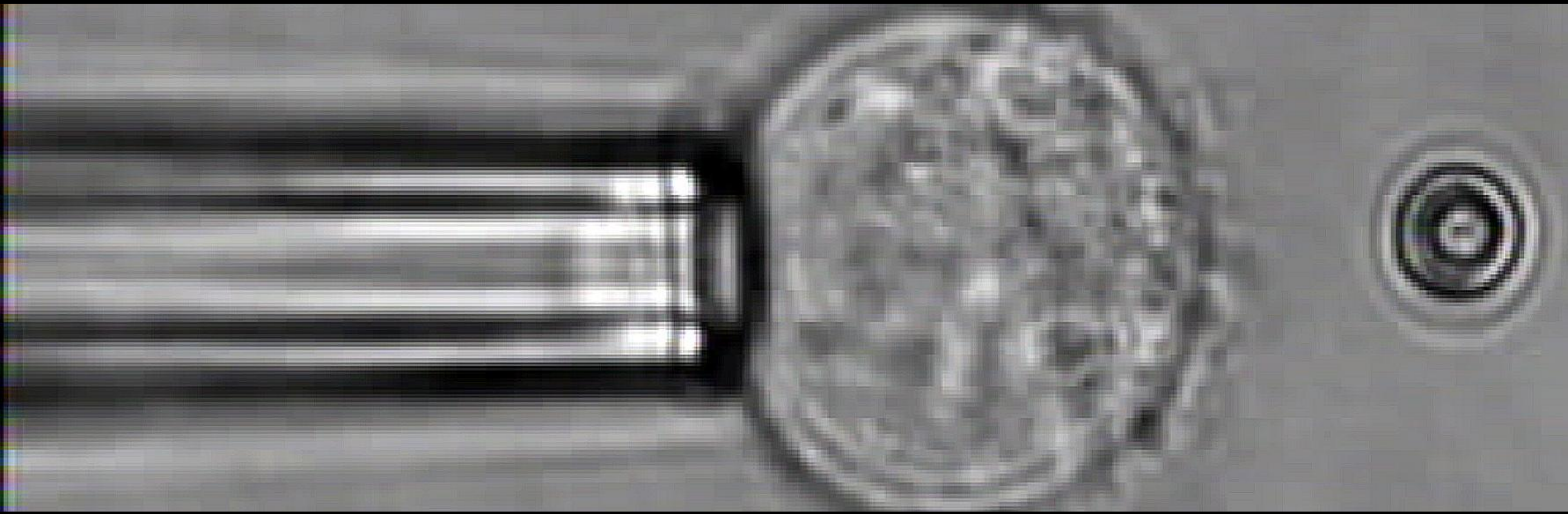
Micropipette aspiration



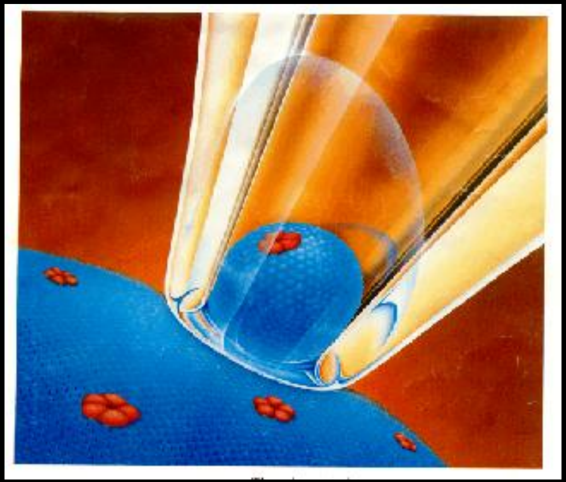
Darius V. Köster

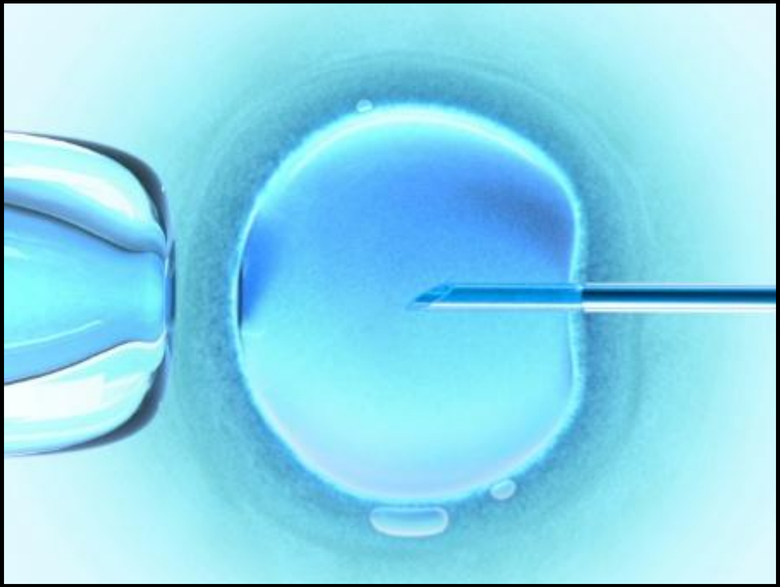
Bangalore, April 25, 2013

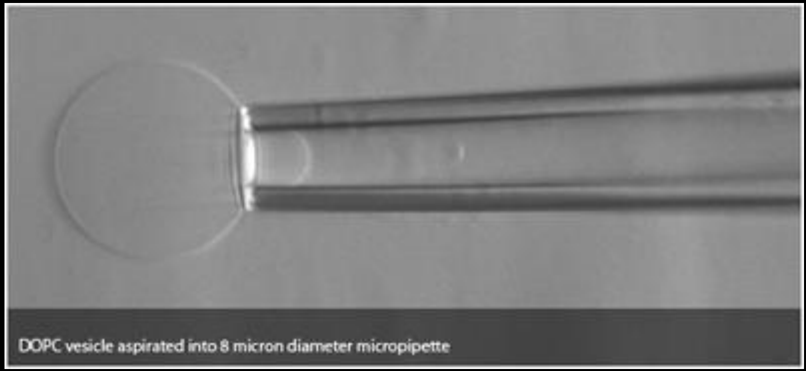
ncbs

Popular applications of micropipette aspiration

- Patch clamp
- Cell injection
- Vesicle aspiration







Imperial college, UK

Key elements of a micropipette system

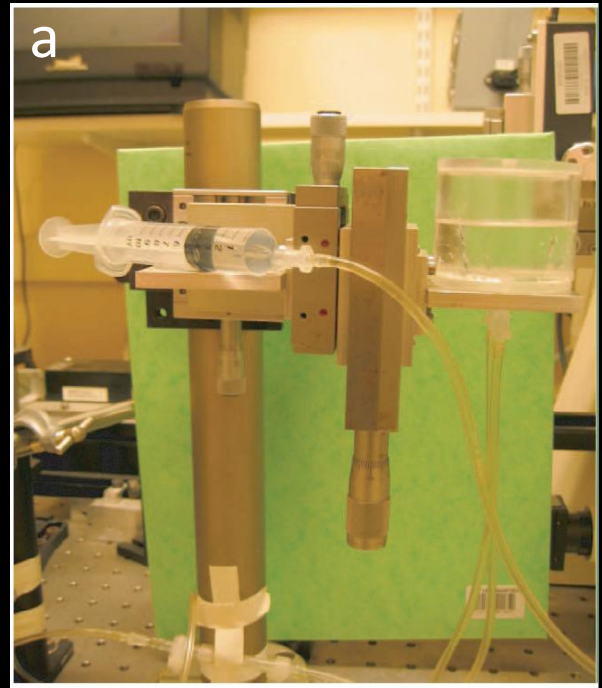
•Pipette: borosilicate glass, 1.0-1.5mm outer diameter

•Pipette holder: tight connections!

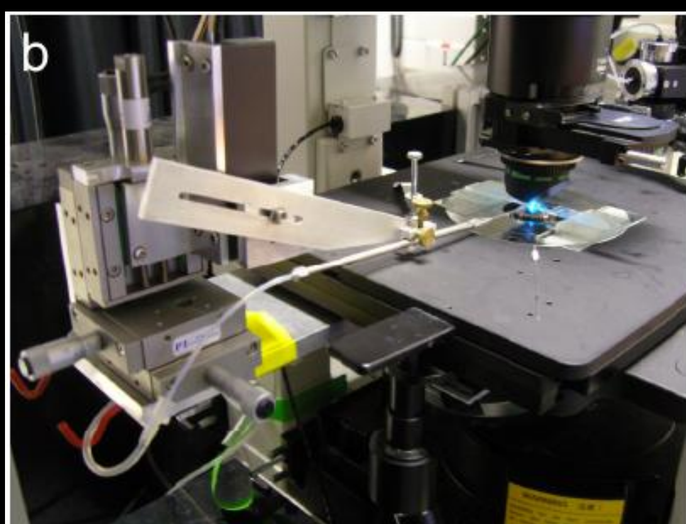
•micromanipulator

•Tubing and valves: flexible plastic, tight connections!

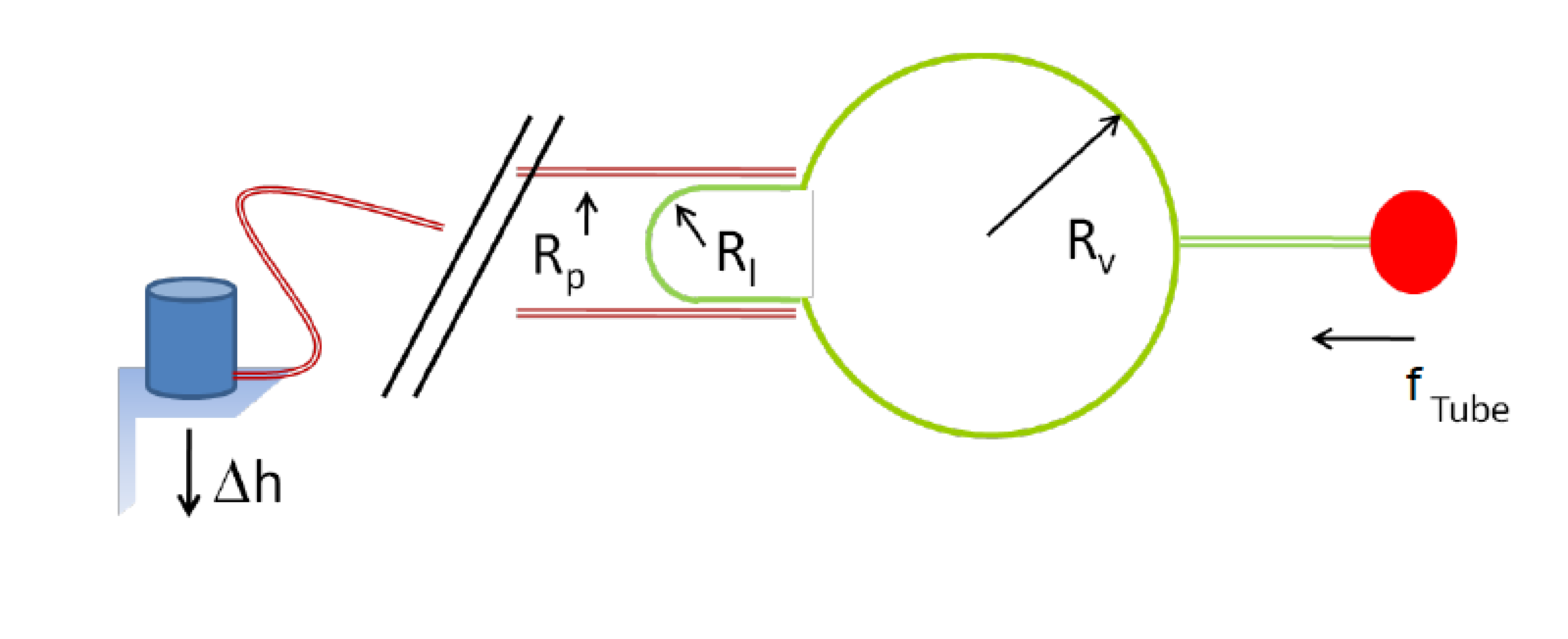
•Pressure control: Vessel + height adjustment/ syringe/ pneumatic system



a) Pressure control
b) Micromanipulator
c) Pipette holder



Principles of micropipette aspiration of vesicles



Set pressure difference at pipette

$$\Delta P = \rho g \Delta h$$

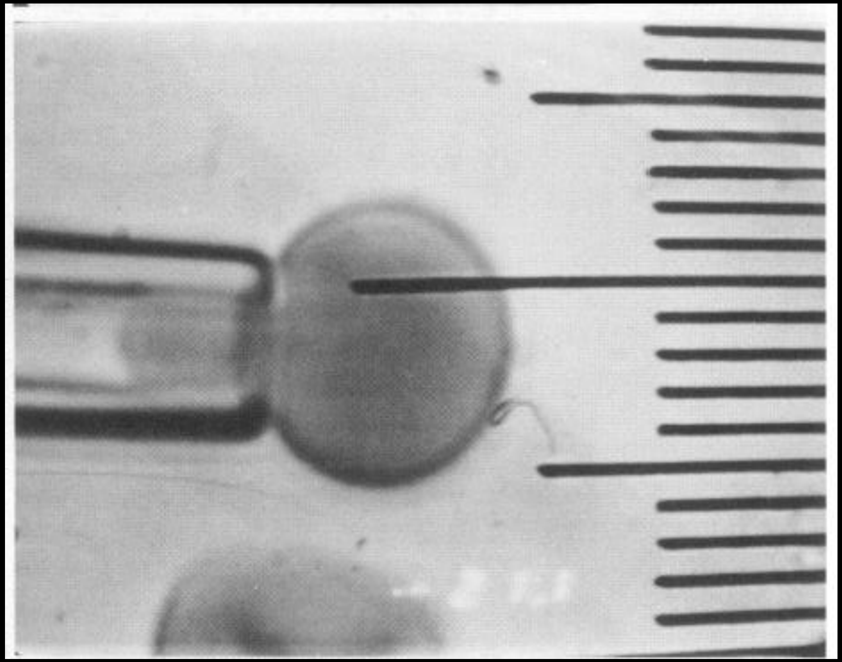
Laplace law

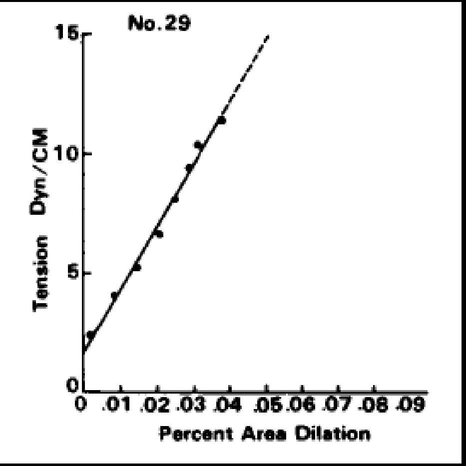
$$\sigma = \frac{\Delta P R_p}{2(1 - \frac{R_l}{R_v})}$$

ELASTIC AREA COMPRESSIBILITY MODULUS OF RED CELL MEMBRANE

E. A. EVANS, R. WAUGH, *and* L. MELNIK

Biophysical Journal, Vol 16, 1976





$$T_o = (K_N + K_{BL}) \cdot \Delta\alpha$$

$K_{BL} \simeq 95$ dyn/cm; (= nN/µm)

$K_N \simeq 193$ dyn/cm.

Relative access area is coupled to bending rigidity κ and the membrane extension modulus K_a (Evans and Rawicz [1990], Rawicz et al. [2000], Fournier et al. [2001])

set

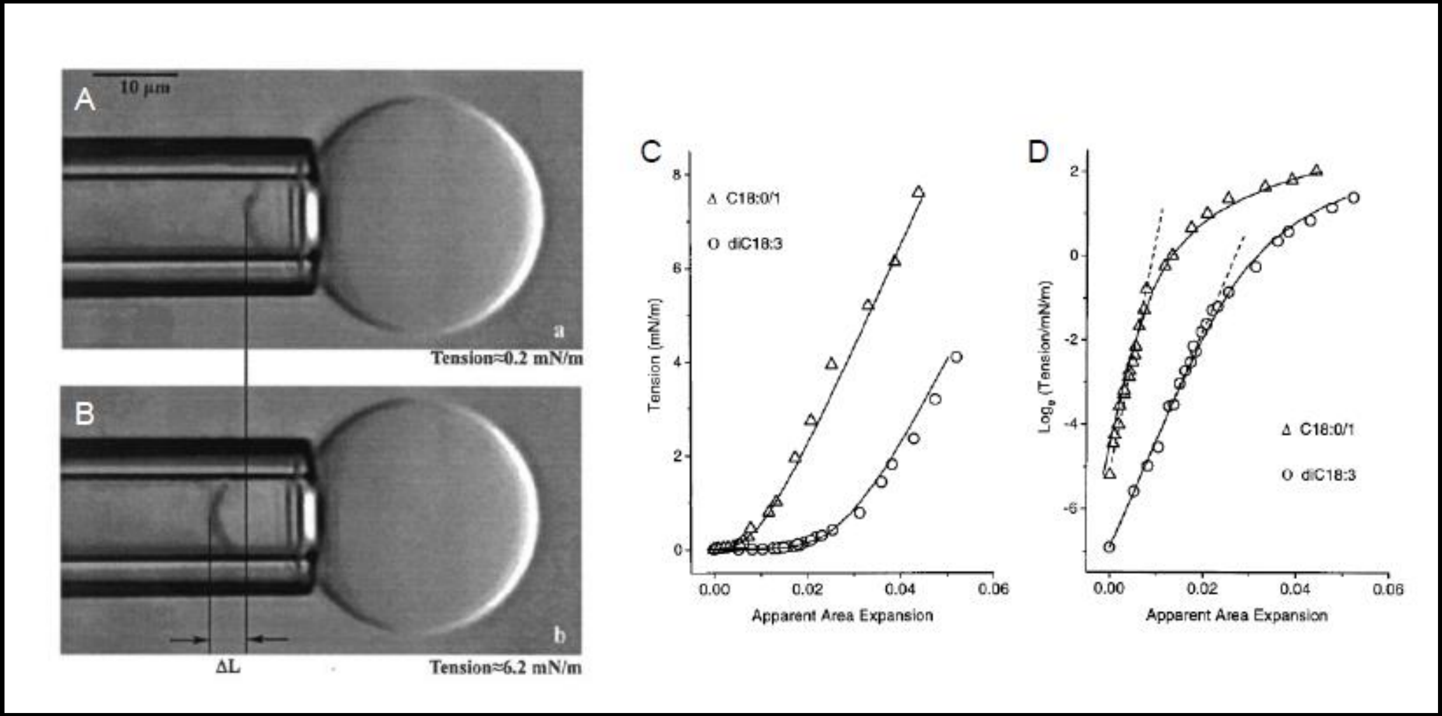
$$\sigma = \frac{\Delta P R_{pip}}{2(1 - R_{pip}/R_{ves})}$$

to measure

$$\frac{A_{asp} - A_0}{A_0} = \frac{(R_{pip}/R_{ves})^2 - (R_{pip}/R_{ves})^3}{2R_{pip}} \Delta L$$

to deduce

$$\alpha_0 - \alpha = \frac{k_B T}{8\pi\kappa} ln\frac{\sigma}{\sigma_0} + \frac{\sigma}{K_a}$$



Membrane Tethers Formed from Blood Cells with Available Area and Determination of Their Adhesion Energy

Robert M. Hochmuth and Warren D. Marcus

Biophsical Journal, 2002

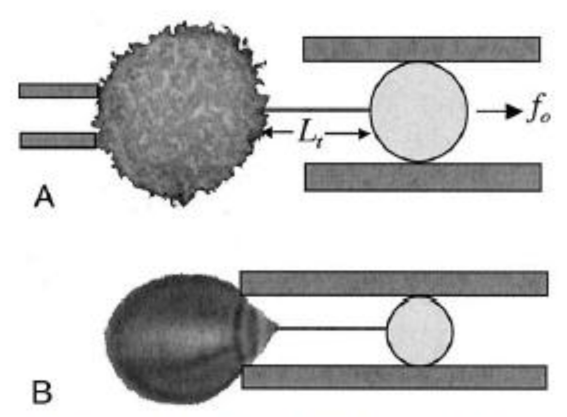


FIGURE 1 (*a*) Membrane tether extracted from a neutrophil stuck to a pipette. The tether force at equilibrium (zero velocity) is f_o and the tether length is L_t. (*b*) Membrane tether extracted from a red cell stuck to a surface.

Neutrophils: 130 pN/µm
Red blood cells: 60 pN/µm

Relation between tether force and adhesion energy of lipid to cytoskeleton

1) $$\frac{f_o}{2\pi R_t} = \gamma_t + \frac{B}{2R_t^2}$$

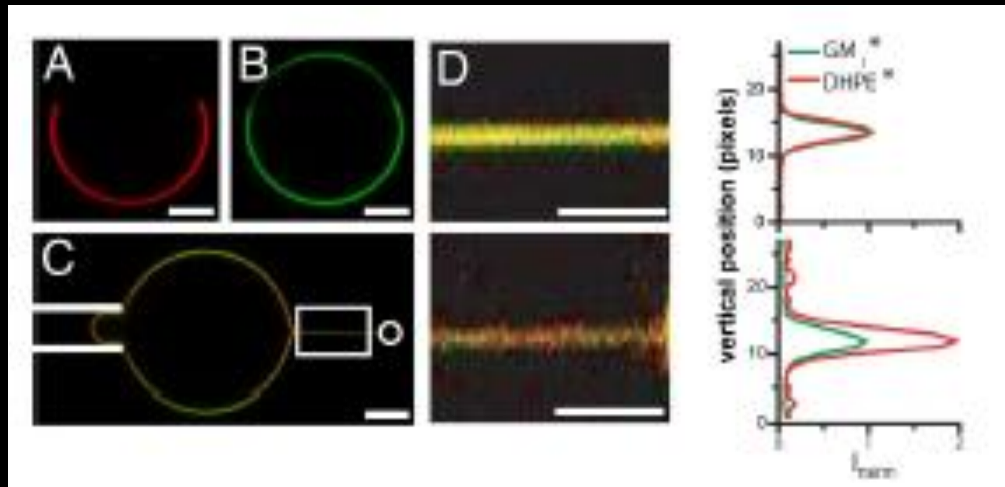
2) $$f_o = \frac{2\pi B}{R_t}$$

3) $$\gamma_t = \frac{f_o^2}{8\pi^2 B}$$

Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins

Benoit Sorre[a,b,1], Andrew Callan-Jones[a,1], Jean-Baptiste Manneville[b], Pierre Nassoy[a], Jean-François Joanny[a], Jacques Prost[a,c], Bruno Goud[b], and Patricia Bassereau[a,2]

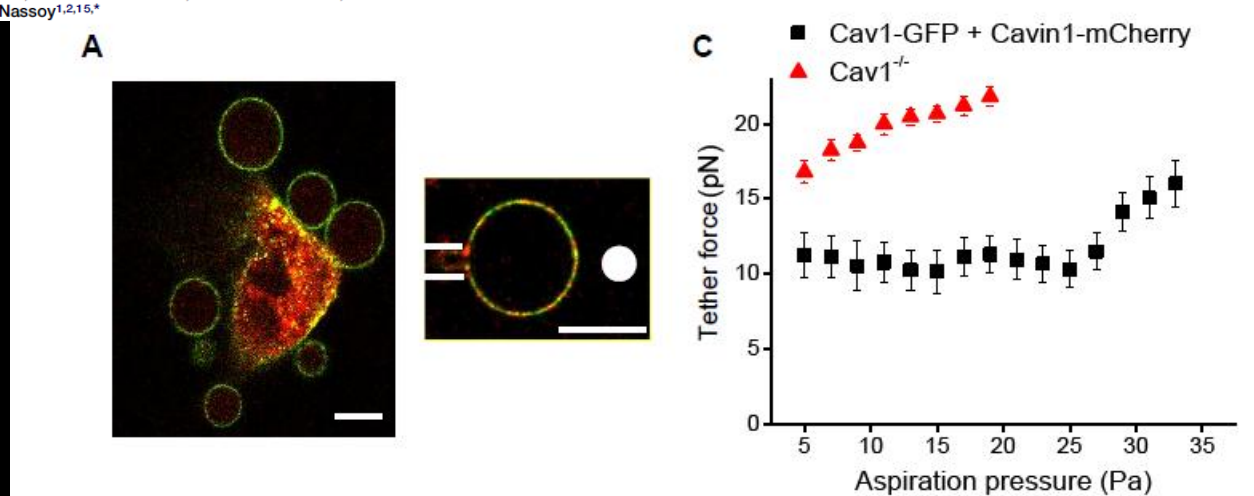
PNAS, 2009



Cells Respond to Mechanical Stress by Rapid Disassembly of Caveolae

Bidisha Sinha,[1,2,14] Darius Köster,[1,2,14] Richard Ruez,[3,4] Pauline Gonnord,[3,4] Michele Bastiani,[8,9] Daniel Abankwa,[8,9] Radu V. Stan,[10] Gillian Butler-Browne,[11] Benoit Vedie,[12] Ludger Johannes,[3,4] Nobuhiro Morone,[13] Robert G. Parton,[8,9] Graça Raposo,[3,5,6] Pierre Sens,[7] Christophe Lamaze,[3,4,15,*] and Pierre Nassoy[1,2,15,*]

Cell, 2011



Alterations in the Young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage

Wendy R. Jones[a,b], H. Ping Ting-Beall[c], Greta M. Lee[d], Scott S. Kelley[d], Robert M. Hochmuth[a,c], Farshid Guilak[a,b,c,*]

Journal of Biomechanics1999

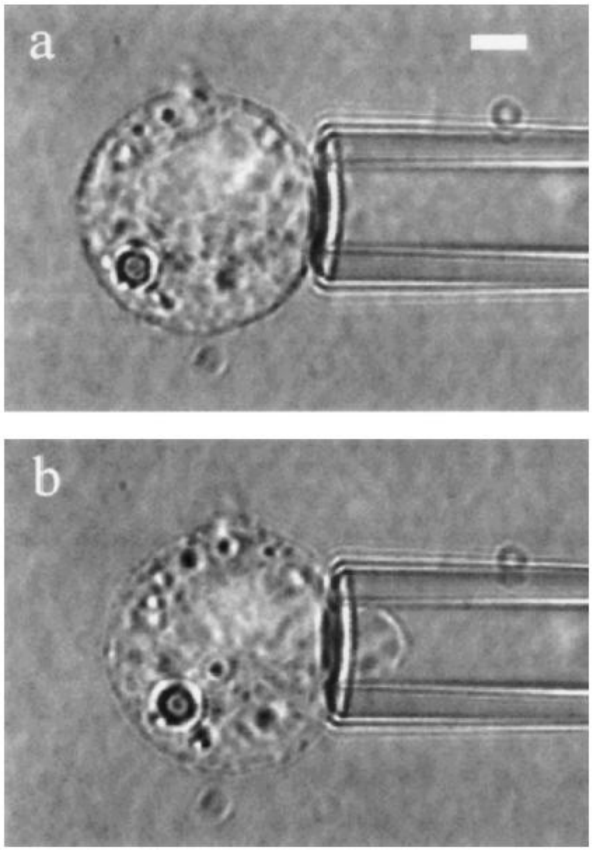


Fig. 2. Micropipette aspiration test to determine the Young's modulus. Application of tare pressure (a) and incremental pressure application (b). Cell projections in the micropipette reached approximately two times the radius of the micropipette, and pressures reached a maximum of 1 kPa. The scale displayed is 5 μm in length.

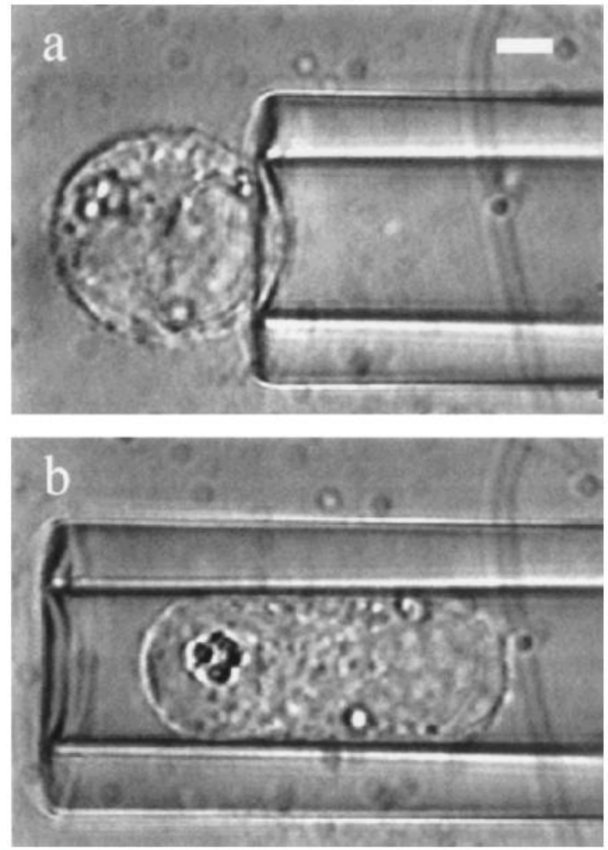


Fig. 4. Micropipette aspiration test to examine volumetric response to mechanical deformation. Video images of a chondrocyte and micropipette before (a) and after (b) complete aspiration of the cell. The scale displayed is 5 μm in length.

Dynamic instability of the intracellular pressure drives bleb-based motility

Benoît Maugis[1,2], Jan Brugués[3], Pierre Nassoy[1,2], Nancy Guillen[4,5], Pierre Sens[3] and François Amblard[1,2,*]

Journal of Cell Science, 2010

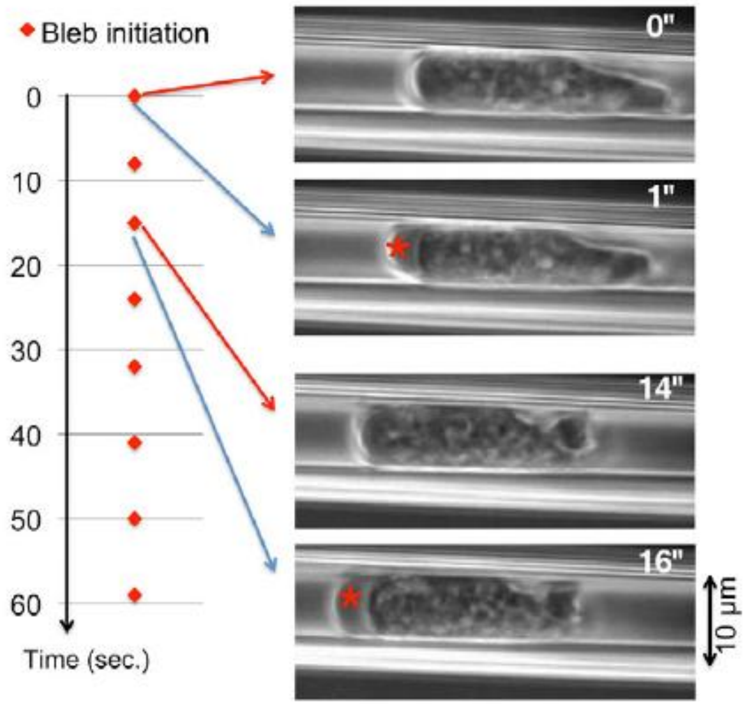


Fig. 7. Blebs become periodic in a static geometry. Using a low suction pressure (500 Pa), *Eh* cells were induced to move inside a 10-µm diameter micropipette. Saltatory motions were observed in time-lapse, phase-contrast videos (see supplementary material Movies 1–13). Successive membrane disjunctions (red asterisks) are clearly resolved in time. These transitions are illustrated by two pairs of images (0/1 seconds and 14/16 seconds). The time course of these transitions (red dots on the time axis) exhibits a clear periodicity of 8±2 seconds (n=46).

To Conclude

- Versatile tool
- Easy to install
- Compatible with many other manipulation techniques
- Compatible with various microscope techniques

THANKS!